REMARKS

Claims 27-69 are pending, and applicants have requested continued examination (RCE).

Applicants thank the Examiner for withdrawing the rejections, under 35 U.S.C. §§ 102 and 103, over Gonzalgo et al, and further in view of Ahern, Whitcombe et al., and Whittwer et al.

The Examiner's new matter rejections of claims 35, 37, 46, 48, 58 and 60, and of claims 27-69, under 35 U.S.C. § 112 ¶1 is acknowledged, and applicants, while maintaining prior arguments, have nonetheless responded to this rejection cancellation of claims 37, 48, 60 and 69.

The Examiner's rejection under 35 U.S.C. § 112 ¶2 for indefiniteness is acknowledged, and applicants have responsively amended *independent* claims 27, 38, 50 and 61 to further clarify the subject matter.

The Examiner's maintained rejections under 35 U.S.C. 103(a) for obviousness, respectively are acknowledged, and applicants have respectfully traversed these particular bases of rejection, but have nonetheless amended the claims to clarify the subject matter.

The Examiner's rejections under obviousness-type double patenting are acknowledged, and applicants reaffirm their willingness to timely file a Terminal Disclaimer upon indication of allowable subject matter.

No new matter has been added.

35 U.S.C. § 112 ¶1 Rejections (New matter)

The Examiner has maintained the rejection of *Dependent* claims 35, 37, 46, 48, 58 and 60 under 35 U.S.C. § 112 ¶1, as containing new matter. Specifically, the Examiner maintains that the Specification does not describe "molecular beacon-type probes," or "scorpion-type primers" (*see* Office Action of 13 January 2004, at page 2, paragraph 4).

Applicants respectfully maintain traverse this rejection, based on the arguments already of record. Molecular beacon probes and scorpion-type primers (comprising intramolecular extension product probes) are art-recognized FRET probes, and were recognized as such in the art at the

time of filing, and particularly in the context of LightCycler® technology. Significantly, LightCycler® technology and Sunrise® Technology were *explicitly* recited in applicants' originally filed Specification in relation to FRET probes (*see* argument of record; applicants' Response and Amendment of 26 September 2003; citing the Specification at page 8, lines 18-20, page 15, lines 24-26, and page 16, lines 7-19; and citing Tygai & Kramer Nature Biotechnology 14:303-308, 1996; Foy & Parkes, *Clinical Chemistry* 47:990-1000, 2001, *referencing*, for Molecular Beacons, Tygai & Krame, *Nature Biotechnology* 14:303-308, 1996, teaching real-time applications of Molecular Beacons; and *referencing*, for Scorpion-type probes; and Whitcombe et al., *Nat. Biotechnol.* 17:804-807, 1999, teaching real-time applications of Scorpion-type). Therefore, applicants' originally filed Specification did more than merely 'discuss dual-labeled FRET probes," as asserted by the Examiner, but disclosed a broader range of probes and probe-probe and primer-probe combinations (Specification at page 15, lines 23-29). Significantly, for example, Whitcombe et al. describe comparing Scorpion primers against two examples of dual labeled probes; namely TaqMan® and Molecular Beacon®, which are grouped together as exemplary dual-labeled FRET probes.

Applicants, to facilitate the present prosecution, have nonetheless herein cancelled dependent claims 37, 48, 60 and 69, which recite Scorpion-type primers.

Applicants, therefore, respectfully request withdrawal of the Examiner's above-identified new matter rejection.

The Examiner has additionally rejected claims 27-69, under 35 U.S.C. § 112 ¶1, as containing new matter. Specifically, the Examiner maintains that the Specification does not describe a "genus of conformational changes" (*see* Office Action of 13 January 2004, at page 4, paragraph 5).

Applicants have responsively amended *independent* claims 27, 38, 50 and 61 to delete the "conformation" language, and to rather recite "detecting the methylated nucleic acid based on at least one of amplification-mediated probe displacement, and amplification-mediated change of

probe fluorescence."

Support for the amendment is found throughout the Specification which discloses the use of FRET probes, and in addition to describing hydrolysis probes (*e.g.*, TaqMan probes), describes general FRET detection based on "amplification mediated probe fluorescence" (*e.g.*, see page 15, lines 21-29). Hydrolysis FRET probes are disclosed as a preferred embodiment (*Id*).

Applicants, therefore, respectfully request withdrawal of the Examiners above-identified new matter rejection of claims 27-69, in view of amended *independent* claims 27, 38, 50 and 61, from which the other rejected claims depend.

35 U.S.C. § 112 ¶2 Rejection

Claims 27-69 were rejected by the Examiner under 35 U.S.C. § 112 ¶2 as being indefinite. Specifically, claims "27-59" [assume 27-69] are alleged to be indefinite over alleged duplication of recitation of "an amplification-mediated displacement or conformation change," and over the recitation "or in a property thereof in relation to another probe or primer" where it is unclear what is being compared (*see* Office Action of 13 January 2004, at page 5, paragraph 6).

As described above in relation to the Examiner's 35 U.S.C. § 112 ¶1-based rejection, applicants have responsively amended *independent* claims 27, 38, 50 and 61 to delete the "conformation" language, and to clarify the claimed subject matter by reciting "detecting the methylated nucleic acid based on at least one of amplification-mediated probe displacement, and amplification-mediated change of probe fluorescence."

Support for the amendment is as describe above.

Applicants, therefore, respectfully request withdrawal of the Examiners above-identified indefiniteness rejection of claims 27-69, in view of amended *independent* claims 27, 38, 50 and 61, from which the other rejected claims depend.

35 U.S.C. § 103 Rejections

The Examiner has maintained the rejection of claims 27-30, 36, 50-53, 59, under 35 U.S.C.

§ 103(a), as being unpatentable over Herman et al. (U.S. Patent 6,017,704, Jan. 25, 2000) (*see* Office Action of 13 January 2004, at page 6, paragraph 8).

Specifically, the Examiner alleges that Herman teaches: "contacting the sample of genomic DNA with bisulfite"; "amplifying the converted nucleic acid with primers that distinguish between methylated and unmethylated DNA such that at least one oligonuclotide probe is a CpG specific probe [but this is incorrect, because Herman does not teach CpG probes]; and "detecting the methylated nucleic acid based on amplification mediated change or property thereof in relation to another probe or primer" (*Id*, at pages 6-9).

The Examiner further alleges that Herman teaches "amplification is carried out using primers specific for CpG-specific oligonucleotides" (Office Action of 13 July 2004, at page 7), and "***finally*** detecting the methylated nucleic acids (citing col.5, lines 60-67) (emphasis added).

The Examiner alleges Herman teaches that amplified products are preferably identified by sequencing, and further asserts that allele-specific oligonucleotide (ASO) probe detection is among Herman et al's listed means for sequencing the amplified products. According to the Examiner, while Herman does not specifically teach a method involving allele-specific oligonucleotide (ASO) probes, it would have been *prima facie* obvious to one of ordinary skill at the time of filing to have modified the method of Herman by using ASO probes for detecting the amplified products. The Examiner, for purposes of this assertion relies on an analogy between genomic alleles and bisulfite-treated DNA.

Applicants maintain traverse to this rejection based on the arguments of record Herman et al do not teach the required use of CpG-specific probes. Additionally and significantly, as pointed out by the Examiner, Herman does not teach or suggest real time measurement, but rather teaches detection *after* amplification ("***finally*** detecting the methylated nucleic acids"). Whatever sequencing suggestion is being made here by Herman is with post-amplification, cloned DNA, and there is absolutely no teachings or suggestions in Herman et al, alone or in combination, that suggests use of CpG specific probes during amplification as a means to provide a real-time signal to distinguish methylated from unmethlyated DNA.

No where does Herman '146 disclose or suggest the instant inventive use of CpG-specific oligonucleotide probes. The essence of the Herman '146 MSP invention is CpG-specific primers, not probes. The Herman '146 Patent is quite clear on this point. ("The oligonucleotide primers distinguish between modified methylated and nonmethylated nucleic acid." Herman '146 Patent abstract, emphasis added). Therefore, Herman et al actually **teach away** from the present invention by teaching that distinguishing methylated and non-methylated DNA is at the primer level and not at the sequencing level.

As describe herein above, applicants have responsively amended *independent* claims 27, 38 and 50 to recite "detecting, *during* the amplification, the methylated nucleic acid based on at least one of amplification-mediated probe displacement, and amplification-mediated change of probe fluorescence" (emphasis added) to further clarify the real-time aspect of the presently claimed subject matter.

Applicants, therefore, respectfully request withdrawal of this rejection in view of applicants' amended *independent* claims 27, 38, 50 and 61.

Additionally, the Examiner maintained the rejection of claims 31-34 and 54-57, under 35 U.S.C. § 103(a) as being unpatentable over Herman et al. (U.S. Patent 6,017,704, Jan. 25, 2000) as applied to claims 27-30, 36, 50-53 and 59 above, in view of Wittwer et al (U.S. Patent 6,140,054, Oct. 2000).

Specifically, the Examiner alleges that while Herman does not teach using FRET probes to detect allele specific differences, Wittwer et al nonetheless do (see Office Action of 13 January 2004 at page 9, para 9).

Applicants traverse this rejection, based on the arguments above in relation to Herman et al. Additionally, while Whittwer et al may teach the use of FRET probes for allele discrimination, the Whittwer method is only a ***quasi*** real-time method, because the method at its essence is based on monitoring fluorescence as a function of temperature to determine a "PCR product melting curve" (*see*, e.g., column 15, lines 55-58; column 16, lines 1-10). The "generated melting curve is

then compared to the known melting curve for the mutant, normal or polymorphic sequence to determine the sequence of the target nucleic acid" (column 4, lines 14-17). Significantly, in the Whittwer method, the FRET probe pair signal is completely abolished every time the PCR temperature is raised (dissociating and thus separating the FRET pair), and a melting curve must be determined and compared. The sensitivity of the assay gradually increases with repeated PCR cycles as the amount of amplificate (and thus product hybridization target) increase.

The instant methods to not require determination of melting curves as do the claimed methods of Whittwer et al. For example, claim 1 of Whittwer recites, in (c), "monitoring the fluorescence as a function of temperature."

Significantly, there is no teaching or suggestion in the art for real-time detection of methylated DNA using CpG-specific probes.

Applicants respectfully request withdrawal of this obviousness rejection in view of Whittwer.

Additionally, the Examiner has maintained the rejection of claims 35 and 38, under 35 U.S.C. § 103(a) as being unpatentable over Herman et al. (U.S. Patent 6,017,704, Jan. 25, 2000) as applied to claims 27-30, 36, 50-53 and 59 above, in view of Witcombe et al (U.S. Patent 6,270,967, Aug. 2001).

Specifically, the Examiner alleges that while Herman does not teach using TaqMan probes to detect allele specific differences, Witcombe et al nonetheless do (*see* Office Action of 13 January 2004 at page 11, para 10).

Applicants respectfully traverse this rejection, based on the fact that there is no teaching or suggestion in Herman et al, or in Witcombe et al to use CpG-specific probes for real-time detection of DNA methylation. Again, Herman teaches away from such an application by specifying that methylation detection is by means of CpG-specific primers, and not probes.

Signficantly, and contrary to the Examiner's assertions, no where is there a suggestion in the asserted art to combine real-time allele discrimination methods with a methylation assay that is

dependent on methylation detection by means of CpG-specific primers, and not probes.

Applicants respectfully request withdrawal of this obviousness rejection based on the above arguments, and those already of record, in view of Herman et al, and further in view of Whitcombe.

Finally, the Examiner maintained the rejection of claims 61-65, under 35 U.S.C. § 103(a), as being unpatentable over Herman et al. (U.S. Patent 6,017,704, Jan. 25, 2000) as applied to claims 27-30, 36, 50-53 and 59 above, in view of Ahern et al (The Scientist, 9:20, July 1995).

Specifically, the Examiner alleges that while Herman does not teach packaging reagents to make kits, Ahern et al nonetheless do (*see* Office Action of 26 March 2003 at page 15, para 14).

Applicants respectfully traverse this rejections, based on applicants' above respective arguments in relation to Hermans.

Nonetheless, applicants have amended claim 61 to recite "a CpG-specific probe the detection of which, during the amplification of the converted nucleic acid, is based on at least one of amplification-mediated probe displacement, and amplification-mediated change of probe fluorescence...."

Applicants, therefore, respectfully request withdrawal of this obvious rejection with respect to claims 61-65.

Obviousness-type Double Patenting Rejection

The Examiners has maintained the rejection of claims 27-32, 38-43, 50-55 and 61-67 under the judicially created doctrine of obviousness-type double patenting as being unpatentable in view of claims 1-26 of U.S. Patent No. 6,331,393 (December 18, 2001) (see Office Action of 13 January 2004, at page 13).

Applicants are fully prepared to timely file a Terminal Disclaimer upon notification of allowable subject matter.

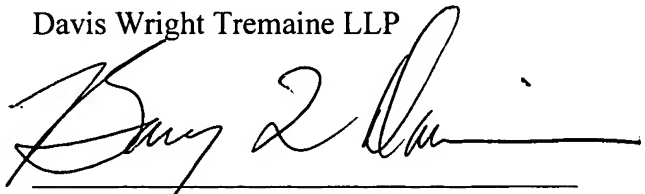
Applicants respectfully request reconsideration and allowance of all pending claims 27-69 of the present continuation application to provide for completion of claims supported by the original specification filed on 14 May 1999 (Issued as U.S. Patent 6,331,393).

No new matter has been added.

Entry of the Amendment is respectfully requested.

Respectfully submitted,

Davis Wright Tremaine LLP



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